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Biosorption of malachite green by novel biosorbent *Yarrowia lipolytica isf7*: Application of response surface methodology

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ABSTRACT

In this study we used a newly isolated *Yarrowia lipolytica* ISF7 strain with a unique capacity to remove malachite green from culture. The effects of operating parameters such as initial pH, dye concentration, temperature and time added on the MG biosorption were studied using response surface methodology, a total of 32 experimental runs were set and the experimental data fitted to the empirical second-order polynomial model of a suitable degree. The optimum biosorption conditions were determined as initial pH 7.0, temperature 25 °C, 24 h and 35 mg L^{-1} of MG concentration. The maximum adsorption capacity for biosorption was found around the pH range 6.5–7.5. Hence from the obtained results it is clear that the noxious dye removal percentage is very low in acidic pH below 5 and alkaline pH above 7.5. The adequate precision (AP) ratio of the models varies between 34.22 for %R MG, which is an adequate signal for the model. AP values higher than 4 are desirable, and confirm that the predicted models can be used to navigate the space defined by the CCD. The nature of biomass dye interactions was evaluated by FT-IR analysis and maximum MG removal (99.889%) was obtained under optimum conditions.

1. Introduction

A negative consequence of the mankind growth and technology has been environmental disorder as large amounts of xenobiotic compounds are accumulated [1]. Limited water resources and increasing demand for safe water make emphasis on development of efficient water treatment methods [2]. Improper treatment and disposal of dye-contaminated wastewaters from textile, dyeing, printing and ink provide serious environmental hazards to most organisms [3,4].

Malachite green (MG) (Table. 1) is extensively used in the aquaculture industry worldwide as a biocide to control external fungal and protozoan infections of fish in addition to its application in the silk, wood, cotton, leather, paper, and acrylic industries [5,6]. This dye may enter into the food chain and could possibly cause carcinogenic and mutagenic effects on humans [7].

The products MG degradation is unsafe due to its carcinogenic potential that makes an urgent and emergency situation for efficient MG removal before discharge into the environment [8–10]. Conventional approaches to remove MG dye include application of activated carbon [11], membrane filters [12], ion exchange [13], chemical coagulation [14], flocculation [15] and biosorption [1,5,16]. These methods are generally effective, but are limited by steep investment costs and generation of toxic sludge [10].

Biosorption possesses good potential to replace conventional treatment methods for dyes and/or metals removal [17]. The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (metal ion and/or dyes) [18].

Yarrowia lipolytica is a dimorphic, non-pathogenic, ascomycetous yeast species with distinctive physiological features and biochemical characteristics that are significant in environment-related matters [19].

In the recent years, *Y. lipolytica* has emerged as an important non-conventional yeast with significant biological relevance and biotechnological applications [20,21]. This yeast has been used in the remediation of various polluted environments [22–24] and is also applied in the degradation of different wastes [25–27]. These literatures encourage us that *Y. lipolytica* as sorbents has good suitability to treat

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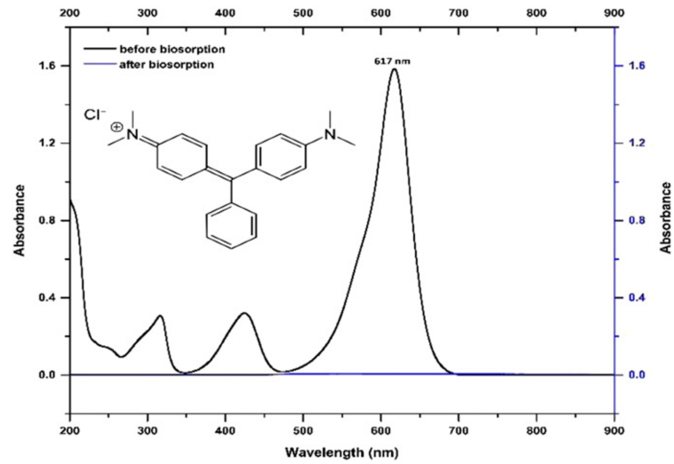
Table 1
Properties of the dyes.

Properties	Malachite green (MG)
Color index number	42000
CAS number	569-64-2
Chemical formula	$C_{23}H_{25}ClN_2$
Molecular weight (g mol^{-1})	364.91
Maximum wavelength (λmax), nm	
Chemical structure	
Absorption spectrum	
Type of dye	Basic yellow (Cationic)

wastewaters as alternative candidate to the conventional treatment methods [28,29].

To the best of our knowledge, there are no reports on the application of this yeast biomass in the removal of dyes, especially MG from aqueous solutions. On the basis of the available literature, we hypothesized that *Y. lipolytica* may be a promising candidate for such biosorption studies.

In this study, response surface methodology (RSM) based on central composite design (CCD) has been used for the optimization of biosorption processes for MG removal by *Y. lipolytica ISF7* (*Y-LISF7*). Important operating variables like pH, temperature, time added and initial concentration of MG that were optimized and evaluated their single and interactive effects of the variables in the process on MG removal efficiency were investigated and their numerical values were calculated. FTIR analysis was conducted to identify the functional groups involved in the biosorption process.

2. Materials and methods

2.1. Materials and instruments

In this work we used the yeast *Y. lipolytica ISF7* (*Y-LISF7*) that isolated from wastewater and registered at NCBI Gene bank with accession number of the strain JX010454.1. Cells were routinely grown at 30 °C on Yeast–Peptone–Glucose (YPG; 1% yeast extract, 2% peptone, 1% glucose, 2% agar medium. YPG broth used for dye biosorption and the pH of medium were adjusted to 7.0 and then were autoclaved (121 °C, 15 min). Malachite green (MG) as a model has net positive charge favorably adsorbed by electrostatic force onto a negative charged surface. MG was supplied by Merck (Table 1). A stock solution of MG (100 mg L^{-1}) was prepared by dissolving 10 mg of analytical grade pure solid in 100 mL distilled water and working solution was provided by serial dilution of above solution. The initial pH in the range of 4.0–8.0 was adjusted by a pH meter (pH-meter model 686 (Switzerland)) via addition of 0.01 M HCl and/or 0.01 M NaOH solution before mixing the biosorbent. A HERMLE bench centrifuge (2206 A, Germany) was used to accelerate the phase separation. The samples were agitated in an incubator shaker (Labcon, FSIM-SPO16, United States) at 160 rpm. The FT-IR spectra of the *Y-LISF7* before and after biosorption of dye were obtained. The transmission FT-IR spectra were then recorded between 400 and 4000 cm^{-1} using Perkin-Elmer (RX-IFTIR, USA) Series FT-IR system. Response surface analysis was performed with the STATISTICA Software (Version 10.0). The significances of all terms in the polynomial equation were analyzed statistically by computing the F-value at a probability (P) of 0.05.

2.2. Batch mode biosorption study

Batch biosorption experiments were carried out in 100 mL Erlenmeyer flasks containing 25 mL of dye solutions (35 mg L^{-1}), time added of 24 h, and pH = 7.0 at 25 °C. The flasks were agitated on a shaker for the desired 24 h. After centrifugation at 3000 rpm for 5 min, the remaining dye concentration was determined using UV–Vis spectrophotometry (617 nm).

Removal efficiency expressed as percent biosorption (R %) of metal ion was determined using the following equation:

$$Percent\ removal.(R\%) = \frac{C_0 - C_e}{C_0} \times 100\% \quad (1)$$

where C_0 and C_e are the initial and final concentrations of dye (mg L^{-1}) respectively.

2.3. Experimental design and optimization

In order to obtain the optimum condition for percentage of MG removal, four independent parameters were selected for the study and are presented in Table 2. The range of study for pH (X_1), initial dye concentration (X_2), time added (X_3) and temperature (X_4) were chosen based on preliminary experiments. The relationship between the parameters and response was determined using central composite design (CCD) under response surface methodology of STATISTICA, a statistical package software version 10.0 (Stat Soft Inc., Tulsa, USA). The CCD design was chosen in this study as it is efficient, flexible and robust. The parameters presented in Table 2 with five levels are coded as −α, −1, 0, +1 and +α, respectively. The total number of experiments obtained for the five factors was ($N = 2^k + 2k + 6$), where k is the number of factors (k = 4) [30]. 32 experiments were conducted with sixteen